

not ferment glucose and maltose. However, *F. tularensis* type A and type B are capable of fermenting glucose and maltose (8). Moreover, *F. tularensis*-specific antigen and antibody tests did not confirm that this strain was *F. tularensis* (9).

Both amplicons of the patient's 16S rRNA gene contained PAEN 515F and PAEN 862F signature sequences. After searching the homologous sequence of the 2 amplicons, the 16S rRNA gene sequence of *P. assamensis* GPT-SA 11 displayed higher homology. Therefore, we concluded that the bacterium isolated from the patient's joint fluid was not *F. tularensis* but *P. assamensis*.

In 2005, a new species of *Paenibacillus* named *P. assamensis* GPTSA 11 was isolated from a warm spring in Assam, India (10). Since then, *P. assamensis* had not been isolated from other environments or patients. Our findings emphasize the need to consider new approaches for preventing infection in the environments where *P. assamensis* exists.

This patient remained at home to recuperate because of his obscure symptoms and financial constraints, but his symptoms did not abate until the follow-up in July 2017. He was advised to return to the hospital for treatment with drugs targeting *P. assamensis*. Isolation of *P. assamensis* from the living and working environments of patients, including soil and water, can successfully reveal the source of infection.

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Plasmodium falciparum Plasmepsin 2 Duplications, West Africa

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Dihydroartemisinin/piperazine (DHA/PPQ) is increasingly deployed as an antimalaria drug in Africa. We report the detection in Mali of *Plasmodium falciparum* infections carrying plasmepsin 2 duplications (associated with piperazine resistance) in 7/65 recurrent infections within 2 months after DHA/PPQ treatment. These findings raise concerns about the long-term efficacy of DHA/PPQ treatment in Africa.

Artemisinin combination therapy has been the cornerstone of malaria control in sub-Saharan Africa for the past 10 years and is typically represented by artemether/lumefantrine and artesunate/amodiaquine. Because of the notorious capacities of *Plasmodium falciparum* to develop drug resistance, many antimalarial programs have recently included dihydroartemisinin/piperazine (DHA/PPQ) as a second-line antimalarial drug. This decision is sensible, considering the recent reports of substantially decreased artemether/lumefantrine cure rates in some regions, signaling a potential focus of lumefantrine resistance (1).

DHA/PPQ has shown near-perfect efficacy levels in clinical trials conducted in Africa; the combination also has been proposed as a tool for intermittent preventive approaches (2). Unfortunately, full *P. falciparum* resistance to DHA/PPQ treatment has been reported recently in Cambodia (3,4). These events were directly associated with increased copy number variations (CNVs) in the plasmepsin system, including the *pfpm2* gene (PF3D7_1408000) coding for the food vacuole enzyme plasmepsin II, which is speculated to be a major piperazine target.

CNV is generally considered as emerging at relatively rapid mutation rates (a rate several orders of magnitude higher compared with that of single-nucleotide polymorphisms [5]) and is able to generate substantial diversity (6). Therefore, preexisting *pfpm2* duplications in Cambodia might have been rapidly selected by DHA/PPQ, aided by a less effective protective action of the artemisinin derivative (7). Such a scenario suggests that this mutation may already be present in Africa.

To investigate this possibility, we analyzed a subset of archived *P. falciparum* DNA samples from clinical infections, derived from a set of large, multicenter comparative artemisinin combination therapy efficacy trials conducted in West Africa by the West African Network for Antimalarial Drugs (8). These trials, performed during October 2011–February 2016 in Mali, Burkina Faso, and Guinea, had a randomized double-blind design with a 2-year follow-up for monitoring repeated treatment. Here we focus on the DHA/PPQ trial conducted at the village of Bougoula-Hameau in Mali, located ≈350 km south of the capital city of Bamako, near the border with Burkina Faso. The weekly control follow-up for each episode at Bougoula-Hameau was 63 days, and the DHA/PPQ arm involved a total of 224 patients who were ≥6 months of age.

We conducted a pilot study analyzing the 96 recurrent infections associated with the shortest interepisode periods, assuming that this subgroup, among whom initiation of recurrent infection ranged from 23 to 65 days posttreatment (Figure), would be the most likely to include *pfpm2* duplications. The study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Pharmacy, and Odonto-Stomatology, University of Sciences, Techniques and Technology of Bamako.

We determined copy number by using a SYBR-green–based quantitative PCR (ThermoFisher Scientific, Waltham, MA, USA) in a protocol modified from the one previously described by Witkowski et al (4). We used the *P. falciparum* β -tubulin gene as the internal nonduplicated standard and the 3D7 clone as a parallel 1 copy control. We ran the quantitative PCR thermal cycle at 98°C for 3 min, followed by 45 cycles at 98°C for 15 s, 63°C for 20 s, and 72°C for 20 s on a C1000 Thermal Cycler (Bio-Rad, Marnes-la-Coquette, France) with the CFX96 Real-Time System (Bio-Rad) detection system. We executed all procedures in triplicate.

The analysis was conclusive in 65 of the 96 samples. We confirm the presence of 7 infections carrying 2 copies of *pfpm2*, representing ≈10% of the successfully analyzed infections. We did not identify any trend of earlier recurrence associated with this group of infections (Figure), a preliminary observation that needs to be further explored in a larger sample set.

Our results clearly show that piperazine resistance–associated *pfpm2* duplications are probably already frequent in Africa, which is of concern given the long half-life of piperazine (>20 days). In high-transmission areas, this long period of decreasing drug exposure is likely to progressively select less sensitive, potentially *pfpm2* CNV–carrying parasites. Parallel studies conducted in these areas have not detected substantial altered parasite clearance dynamics or K13 mutations associated with artemisinin-derivative therapy (9,10), indicating that these *pfpm2* duplications are emerging despite the efficacy of dihydroartemisinin. Further studies are urgently needed to clarify the clinical implications of piperazine resistance and to monitor occurrence in other areas of high malaria transmission in Africa.

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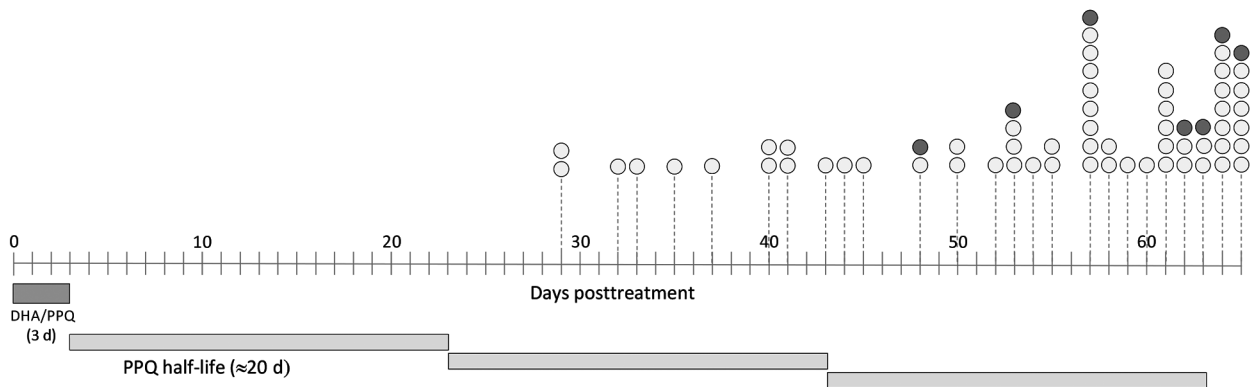


Figure. Timeline distribution of *Plasmodium falciparum* *pfpm2* copy number status during post-DHA/PPQ treatment followup for artemisinin combination therapy efficacy trials conducted by the West African Network for Antimalarial Drugs, Mali, Burkina Faso, and Guinea, October 2011–February 2016. Dark gray bar highlights the period (3 d) of treatment; lighter, longer gray bars represent PPQ average half-life (≈ 20 d). Circles represent recurrent infections; white circles indicate 1 *pfpm2* copy, and gray circles indicate 2 *pfpm2* copies. DHA/PPQ, dihydroartemisinin/piperazine; PPQ, piperazine.

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